commentary

The ceramide ratio: a predictor of cardiometabolic risk[1]

Trevor S. Tippetts, William L. Holland, and Scott A. Summers[2]

Department of Nutrition and Integrative Physiology, University of Utah College of Health, Salt Lake City, UT 84112

Circulating lipids drive the tissue dysfunction that underlies cardiovascular disease and diabetes. Clinical indices of risk of these metabolic disorders include serum levels of LDLs, total and LDL-cholesterol, and triglycerides, all of which reveal heightened susceptibility for major adverse cardiac events (MACEs). Despite their widespread use, these established clinical biomarkers only weakly forecast cardiovascular outcomes, leaving substantial need to develop more reliably predictive diagnostic tests. In this issue of the *Journal of Lipid Research*, Anroedh et al. (1) provide evidence supporting a new serum diagnostic: the C16/C24 ceramide ratio.

Ceramides are synthesized from dietary metabolites or products of fatty and amino acid metabolism through a four-step biosynthetic cascade, with the final products containing a sphingoid base coupled to variable acyl side chains via amide linkage. Six distinct (dihydro)ceramide synthases (*Cers1–6*), which differ by tissue location and substrate specificity, facilitate addition of the acyl chains to the sphingoid backbone to produce the diverse pool of ceramide species (2). These ceramides can be further metabolized by enzymes that add different head groups to produce the complete spectrum of tissue sphingolipids (e.g., sphingomyelins and glycosphingolipids). A recent flurry of studies has revealed clinical utility for measuring the ratio of certain ceramides, particularly the long-chain C16:0 ceramide, in relation to very-long-chain C24:0 ceramide. Indeed, high 16/24 ratios show a strong association with MACEs, as well as other metabolic defects that contribution to the pathology (e.g., coronary artery disease, insulin resistance, etc.) (3–6). Other ceramides (e.g., C18:0, C20:0, C22:0, etc.) also associate with these clinical pathologies, but C24:0 does not (3–6).

Through the European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis (ATHEROREMO), Anroedh and colleagues built upon these prior studies, conducting a long-term (5 year) follow-up analysis of 574 plasma samples obtained from older adults (mean age: 61.5 years) with either a diagnosis of acute coronary syndrome (ACS) or stable angina pectoris (SAP). They screened for relationships between cardiovascular outcomes and ten circulating biomarkers, including the ceramide species and ratios described previously. As expected, they confirmed that associations between the aforementioned, well-characterized markers of cardiovascular disease (e.g., high LDL cholesterol, high serum-nonHDL cholesterol, and low triglycerides) were evident in subjects with ACS (as compared with those with SAP). Notably, they also determined that C16:0, C20:0, and C24:1 ceramides were elevated in ACS patients. These relationships were particularly robust when presented as a ratio against C24:0 ceramides, which showed no such association with MACEs (1). By employing a multivariate analysis, the authors controlled for gender, age, hypertension, hypercholesterolemia, diabetes mellitus, and statin use. After further controlling for either LDL cholesterol or nonHDL cholesterol, the association between C16:0 ceramides and MACEs persisted (1). When the authors investigated secondary endpoints (nonfatal ACS, all-cause mortality), similar associations between ceramide ratios (e.g., C16:0/C24:0, C18:0/C24:0, and C24:1/C24:0) and disease endpoints were revealed (1).

These latest studies complement a number of recent clinical investigations reporting associations between ceramides and cardiovascular outcomes, to the extent that the Mayo Clinic is marketing diagnostic tests to measure ceramides (5, 7–9). These studies show remarkable consistency, as a subset of ceramides (e.g., C16:0, C18:0, C20:0) almost invariably associates with deleterious outcomes, while the C24:0 ceramides show no or negative relationships. This presents the intriguing diagnostic utility of tests reporting the ratios of the harmful ceramides against the benign C24:0 species.

Studies in rodent models reveal that these ceramides play causative roles in cardiovascular pathologies, eliciting the metabolic impairments that underlie their development. For example, interventional studies in rats, mice, or hamsters that either *a*) slow the synthesis or *b*) stimulate the degradation of ceramides ameliorate atherosclerosis,

The authors receive research support from the National Institutes of Health (DK112826 and DK108833 to W.L.H. and DK115824 to S.A.S.), the Juvenile Diabetes Research Foundation (to W.L.H.), the American Diabetes Association (to S.A.S.), the American Heart Association (to S.A.S.), and the Huntsman Cancer Institute (to S.A.S.). We disclose that Scott Summers is a consultant and shareholder for Centaurus Therapeutics.

DOI https://doi.org/10.1194/jlr.C088377

[1]See referenced article, *J. Lipid Res.* 2018, 59: 1729–1737.

[2]To whom correspondence should be addressed.
e-mail: Scott.a.summers@health.utah.edu

insulin resistance, type 2 diabetes, fatty liver disease, and cardiomyopathy (10). For example, pharmacological inhibition or genetic ablation of the biosynthetic enzymes serine palmitoyltransferase or dihydroceramide desaturase-1 produce robust protective effects in animal models of cardiometabolic dysfunction (e.g., feeding obesogenic diets, leptin or leptin receptor deficiency, apolipoprotein E-deficiency, etc.) (10). Additional studies have probed the relevance of the specific acyl-chains, supporting the importance of the C16/C24 ratio as a driver of disease. For example, genetic deletion of ceramide synthase 6 (*Cers6*), which produces C16:0 ceramides, elicits metabolic improvements (11). By comparison, deletion of *Cers2*, which produces C24:0 ceramides, exacerbates metabolic pathologies (12). Of note, *Cers2* deficiency leads to compensatory upregulation of *Cers6*, further driving the formation of the deleterious C16:0 ceramides. These observations strongly support the therapeutic potential of pharmaceuticals targeting sphingolipids, as well as diagnostic tests using the C16/C24 ceramide ratio as a marker of risk.

The molecular means by which these particular ceramides drive tissue dysfunction has received considerable attention (10). They inhibit activation of the insulin-stimulated, anabolic enzyme Akt/PKB, thus contributing to the insulin resistance that underlies many of these pathologies. They also impair mitochondrial function and drive oxidative stress. Another subset of actions have been shown to relate to their ability to promote retention of lipoproteins in the vessel wall (10). Lastly, they trigger cardiomyocyte-specific actions related to cardiomyopathy (13). Despite these successes, scientists have an incomplete understanding of why ceramide chain-lengths have such potent implications on tissue function. This engaging question will surely be an area of interest and mechanistic inquiry as researchers strive to elucidate the complexity underlying ceramide actions on tissue function.

In summary, the recent application of targeted and untargeted lipidomics has uncovered the exciting diagnostic potential of measurement of serum ceramides as indices of disease risk. They represent an exciting class of molecules of considerable therapeutic potential and clinical utility.

REFERENCES

1. Anroedh, S. S., M. Hilvo, K. M. Akkerhuis, D. Kauhanen, K. Koistinen, R. Oemrawsingh, P. Serruys, R. J. van Geuns, E. Boersma, R. Laaksonen, et al. 2018. Plasma concentrations of molecular lipid species predict long-term clinical outcome in coronary artery disease patients. *J. Lipid Res.* **59:** 1729–1737.
2. Park, J. W., W. J. Park, and A. H. Futerman. 2014. Ceramide synthases as potential targets for therapeutic intervention in human diseases. *Biochim. Biophys. Acta.* **1841:** 671–681.
3. Summers, S. A. 2018. Could ceramides become the new cholesterol? *Cell Metab.* **27:** 276–280.
4. Tarasov, K., K. Ekroos, M. Suoniemi, D. Kauhanen, T. Sylvanne, R. Hurme, I. Gouni-Berthold, H. K. Berthold, M. E. Kleber, R. Laaksonen, et al. 2014. Molecular lipids identify cardiovascular risk and are efficiently lowered by simvastatin and PCSK9 deficiency. *J. Clin. Endocrinol. Metab.* **99:** E45–E52.
5. Laaksonen, R., K. Ekroos, M. Sysi-Aho, M. Hilvo, T. Vihervaara, D. Kauhanen, M. Suoniemi, R. Hurme, W. Marz, H. Scharnagl, et al. 2016. Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes beyond LDL-cholesterol. *Eur. Heart J.* **37:** 1967–1976.
6. Lemaitre, R. N., C. Yu, A. Hoofnagle, N. Hari, P. Jensen, A. M. Fretts, J. G. Umans, B. V. Howard, C. M. Sitlani, D. S. Siscovick, et al. 2018. Circulating sphingolipids, insulin, HOMA-IR and HOMA-B: the Strong Heart Family Study. *Diabetes. Epub ahead of print.* March 27, 2018; doi: 10.2337/db17-1449.
7. Westra, B. 2016. Ceramides, Plasma [A Test in Focus]. Mayo Medical Laboratories. Accessed June 15, 2018 at https://news.mayomedicallaboratories.com/2016/07/28/ceramides-plasma-a-test-in-focus/
8. Meeusen, J. W., L. J. Donato, S. C. Bryant, L. M. Baudhuin, P. B. Berger, and A. S. Jaffe. 2018. Plasma ceramides: a novel predictor of major adverse cardiovascular events after coronary angiography. *Arterioscler. Thromb. Vasc. Biol.*
9. Havulinna, A. S., M. Sysi-Aho, M. Hilvo, D. Kauhanen, R. Hurme, K. Ekroos, V. Salomaa, and R. Laaksonen. 2016. Circulating ceramides predict cardiovascular outcomes in the population-based FINRISK 2002 cohort. *Arterioscler. Thromb. Vasc. Biol.* **36:** 2424–2430.
10. Chaurasia, B., and S. A. Summers. 2015. Ceramides - lipotoxic inducers of metabolic disorders. *Trends Endocrinol. Metab.* **26:** 538–550.
11. Turpin, S. M., H. T. Nicholls, D. M. Willmes, A. Mourier, S. Brodesser, C. M. Wunderlich, J. Mauer, E. Xu, P. Hammerschmidt, H. S. Bronneke, et al. 2014. Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. *Cell Metab.* **20:** 678–686.
12. Raichur, S., S. T. Wang, P. W. Chan, Y. Li, J. Ching, B. Chaurasia, S. Dogra, M. K. Ohman, K. Takeda, S. Sugii, et al. 2014. CerS2 haploinsufficiency inhibits beta-oxidation and confers susceptibility to diet-induced steatohepatitis and insulin resistance. *Cell Metab.* **20:** 919.
13. Park, T. S., and I. J. Goldberg. 2012. Sphingolipids, lipotoxic cardiomyopathy, and cardiac failure. *Heart Fail. Clin.* **8:** 633–641.